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FOREST INSECT INVESTIGATIONS

CONTRIBUTIONS TO THE KNOWLEDGE OF THE LIFE PARTNERSHIP BETWEEN BARK BEETLES AND FUNGI

by

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Contents

Introduction

Statement of Problem, Material and Methods

The Relation of Certain Ipides to Fungi 9

a) Blue-stain Fungi Carried by Ipides 10
b) Yeasts Carried by Ipides 39

General Remarks about Wood and Bark Nutriment 52

Description of Some of the Fungi Found During the Course of the Experiment 54

a) Leptographium penicillatum n. sp.
b) Graphium pycnocephalum n. sp.
c) Graphium adustum n. sp.
d) Dendrostilbella hyalina n. sp.
e) Experiments with the species of yeasts found with Ips typographus 62

Results of the Investigation of the Symbiosis Problem 69

Bibliography

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Introduction

Insects which feed on plant tissues have the contents of the cells and the cell walls to consume.

The cell contents, especially the contents of the parenchyma cells, consist principally of proteins and are also rich in reserve stuffs in the form of reserve proteins, sugar, starch, and fats. These are easily digested by the digestive enzymes of animals.

The cell wall consists of cellulose and incrustations which serve partly for reenforcements, partly as reserve carbohydrates, and often serve both purposes.

The incrustations of the cellulose membranes consist of so-called hemicelluloses, and in the case of woody tissue, also of lignin. Hydrolytic splitting of the hemicelluloses gives partly Hexoses, partly Pentoses, in which one distinguishes Hexosane and Pentosane. The Glucanes belong to the Hexosanes; among them one of the most important is Lichenin — also Mannane and Galactane, to the Pentosanes Xylane and Arabane. The chemical construction of lignin is not entirely understood. It consists of C, H and O in circular bond and cannot be reduced to sugars. It also contains carbohydrate, either in adsorbed or in stored form, which is with difficulty reduced to sugar by the use of concentrated acids. It also contains nitrogen compounds.

An estimation of the value to the insects of cellulose-rich pabulum is fraught with difficulties. Can cellulose or its incrustations

be decomposed directly, when naturally the necessary enzyme in the insect intestines is not present? At any rate, so far it is not definitely known that an insect is able to produce such an enzyme.

Seillere (1905) determined that an enzyme which specifically attacked Pentosane, probably a Xylanase, was in the intestines of the bark beetle larvae (Rhymatodes variabilis L.). These were mining both the splint and bark of beech. He, himself, was doubtful whether his results on the digestion of cellulose were trustworthy, as possibly cellulose-destroying bacteria could have played a part. This restraint appears necessary in accordance with the new viewpoint in symbiotic experimentation.

Buchner (1928) pointed out that in Gastropacha rubi (Macrothylacia rubi L.) the blackberry spider whose caterpillar lives on the leaves of low plants, blackberry, rose, shrubby oak and other shrubs, there is a lichenase. Buchner is inclined to attribute this lichenase to the blackberry spiders themselves. As he states, the fantastic findings of Portiers (1911) on the symbiosis of the Lepidoptera have not been proved, but possibly a symbiosis "is realized in a very limited way." Grandori (1925) believed he had found such a hidden, inherited bacterial symbiosis in Bombyx mori. It also remains doubtful whether the blackberry spider really is symbiotically free and whether the caterpillars themselves produce the lichenase.

An insect dependent on cellulose as food, which has at its disposal a great deal of cell wall and little cell content as, for example, is the case when feeding on wood, if it does not possess the necessary enzymes for digestion, must live in a life partnership with organisms. These will have to be such as are in a position to feed on the cellulose

substratum and change it into a form which can be used as food so that the insect can reach the cell content which has been surrounded by cellulose, or produce by-products of the cell walls which can be used by the animal. Buchner (1928) pointed this out in his lecture on "Wood as Food and Symbiosis." Buchner and his pupils have led, during the last 10 years, the experimental work on Symbiosis. The first step in solving the problem of the use of cellulose as food by cellulose-destroying organisms must be made with the use of wood already infected with fungi or bacteria.

In some families of Clavicornia and Heteromera among which, for example, are the Cisidae, Melandryidae and Mordellidae, there are many species whose larvae live in fungus-infected wood in a thick felt of hyphae under the bark or in the fruits of the fungus. The individual species of beetles usually are associated with special kinds of fungi, all belonging to Hymenomycetes and known as wood destroyers. If, in some cases, there is a narrower, interchangeable relation between beetles and fungi, they have not as yet been studied.

Such interrelation may be a higher form of interdependence of insects with cellulose digesting organisms, a "reciprocal relationship" in which both partners have their own advantages, in other words a symbiosis. If one uses the term symbiosis with a wider meaning, it is not necessary, that both parties are absolutely necessary to each other, it must be the optimal way of life for both or at least for one partner to have this community life.

The advantage for an organism which is capable of living on wood in a community life with an insect consists in its distribution or its cultivation by the insect or even its storage in the body of the insect.

The advantage for the insect in a community life with a cellulose digesting organism can be thought out in different ways. The insect feeds on the substance of his symbiot or he uses its enzymatic or assimilative ability.

The first case deals, for example, with an outside mutual dependence with fungi in which the insect cultivates the fungus on a cellulose-rich substratum which has been either carried artificially or is there naturally and uses it as food; in other words, a conversion of the indigestible part of the substratum and the inaccessible cell contents into fungus food. In this case there is also the possibility of an inner symbiosis; namely, the symbiot may be cultivated and protected in the stomach.

The second case can be thought of as a using of the decomposition of the wood particles produced by the ectoenzyme of the symbiotic organisms, which live freely in the stomach lumen or intracellularly in special organs in the interior of the insect, or as using the products of secretion in the symbiotic organs.

As a result of these possibilities the insects can be seen to solve the problem of using cellulose in different ways. The termites cultivate fungus on a substratum which they make of cellulose-rich material, of small pieces of leaves, or rotting wood, just as the Atta ants. The cultivation of ambrosia is a case of fungus cultivation on a natural cellulose-rich substratum. This is done by the Lymexylides, Hylecoetus dermestoides and bark beetles breeding in wood.

The circumstances of inner symbiosis are less clear. The termites which do not cultivate fungi carry protozoa in pockets of their intestines. These apparently are concerned in the digestion of cellulose. These intestinal pockets filled with cellulose fermenting bacteria, called by Buchner "fermentation rooms", are found in the larvae of some Diptera that feed on wood or compost and also some Lamellicornier having the same mode of living.

No experiments have been made so far to explain the working of intracellular symbiosis. This has been known for some time in the Anobiae and has lately been ascribed by Buchner and Heitz to several Buprestids, Curculionidae and Cerambycids. It consists in this instance of yeasts (Anobiae, Cerambycids) or bacteria-filled organs which are connected with the intestinal canal of larvae (Curculionidae, Buprestidae), and sometimes also of imagoes. Buchner believed he saw in these intracellular symbioses an equivalent to ambrosia cultivation, and connected these symbioses directly with cellulose digestion. He was supported by the fact that the larval organs disappear in the imagoes of the Stag beetles and weevils, which live on the sap of plants, but there is a special apparatus carried over for the infection of the future wood-feeding generation. Heitz emphasizes the fixation of free nitrogen by the yeast organs, known to take place with some species of Torula. A conclusion about the feasibility of intracellular symbiosis can be reached only by physiological experiments.

Statement of Problem, Material and Methods

Different kinds of symbioses are found among the Tetramera: intracellular bacterial symbiosis of the Curculionidae, intracellular yeast symbiosis of the Cerambycidae, and ambrosia cultivation by the wood-boring Ipidae.

There is another group of Tetrameren, the bark-breeding Ipidae, in company with which there is another rich fungus flora and in addition very frequently a vigorous fungus vegetation of the insect's stomach. Here may be a symbiosis to investigate, possibly with fungi, and leading from this consideration, the characteristics of the fungi associated with the Ipides and their importance in the ecology of the beetles. These questions are of interest to foresters in so far as some of these fungi are known as the cause of plant diseases (the Dutch elm disease), and others as detrimental to forest products (blue stain).

This paper deals with the questions: Whether fungi accompany the Ipides which breed in the bark, and whether an outer symbiosis exists such as that of the ambrosia cultivators or an inner symbiosis such as that of the Cerambycids.

I am indebted to Professor H. Prell, my teacher, for undertaking this work. I wish to thank Professor E. Münch for his valuable advice and help in carrying on the work. Assistant W. Baer determined the beetles; also, Assistant Fredrick helped photograph.

I am grateful for the material sent by Forester Schönfelder of Hinterhermsdorf, Swiss Saxony; Forester Schröter, Postelwitz, Swiss Saxony;

Forester Umrath, Klosterreichenbach, Württemberg, and Professor Prell for giving me Scolytus material from his garden in Loschwitz.

The research was carried on principally with Ips typographus L. I had a large amount of material from Postelwitz, Hinterhermsdorf, Swiss Saxony, and for comparison with a region having different geographical characteristics, from Klosterreichenbach district (Württemberg). I also worked with Blastophagus piniperda L. which had killed the trap trees in the Tharandt Forest. For comparative work I used material from Spechtshausen, Hinterhermsdorf, and Ottendorf-Okrilla.

Besides, work was undertaken with Scolytus scolytus Fabr. and Scolytus pruni Rtzb. (Dresden-Loschwitz), Hylurgops palliatus Gyll., Polygraphus poligraphus L., Crypturgus cinereus Herbst, Dryocoetes autographus Rtzb., Ips amitinus Eichh., Onthotomicus laricis Fabr., Pityogenes chalcographus L. (Hinterhermsdorf, Postelwitz, Tharandt).

Research on the flora outside the insects was made by using the fungi in the bark, wood and frass isolated according to the usual methods. The pure cultures are single spore cultures, which were procured by the shake method and many dilutions.

For studying the fungus flora of the intestines and the spores which had clung to the insect, the cultures were procured by the shake method using the stomach contents, and the washings of insects, plated in cleared malt gelatine. To prevent bacterial contamination the medium was acidified with 4 per cent citric acid. This method allowed me to study the growths with the microscope and isolate single fungus spores and colonies.

The outside of the stomachs of the insects were sterilized by putting them in 1 per cent mercury bichloride solution for 1 minute, and rinsing frequently in tap water. The stomach was handled with flamed instruments, crushed, and the contents washed out with a small amount of tap water. Erlenmeyer flasks containing melted malt gelatine was inoculated with the washings and the medium poured into Petri dishes.

The fixation of the materials was made with mixtures of Flemming, Zenker and Schaudinn. The sections were dyed with Heidenhain's Hämatoxylin and eosin or Hämalaun-Orange G. The latter stain gave the best results for the stomach flora. It produced a differentiation with iron alum in which the yeast cells were blue, the animal tissue orange with brown nuclei.

The Relation of Certain Ipids to Fungi

When examining the frass of bark-breeding Ipids, one finds two very different groups of fungi present. On the one hand are the so-called blue-stain fungi, which cause a more or less deep discoloration of the wood. On the other hand there are some forms of fungi which are closely related, but cause very little discoloration of their substratum. The appearance of blue stain is especially noticeable around the galleries on tree trunks freshly infested with Ipids. It is less apparent around the insect galleries on logs which have been infested for a longer time and have winged beetles.

The second group are yeasts which proliferate in the frass and often can be recognized as a smeary, glistening mass.

The problem was to give the characteristics of the fungi which accompany Ipids, their transferrence by the beetles, their spread, and finally to investigate their importance for the insects.

a) The Blue-stain Fungi Carried by Ipides

First the appearance of blue stain will be discussed, and I shall also give a general discussion of blue stain and a resume of the important literature.

By blue stain one understands the discoloration of wood into more or less dark shades of blue, gray green or blackish colors which are caused by different fungi. The fungi belong partly to the Ascomycetes, partly to the Fungi imperfecti. Among the latter are the Sphaeropsides, several species of which deeply color the substratum in which they grow; for example, species belonging to the Excipulacae Sacc., the family Discula Sacc. The Hyphomycetes also have many blue-staining fungi. In the family of the Dematia Fries are found the genus Hormiscium Kunze, Hormodendron Bonord, and Alternaria Nees, a number of wood-discoloring fungi. Of the Stilbaceae Fries, the genus Graphium Corda has many forms of blue stainers.

The Ascomycetes are represented by many species of Pyrenomycetes, the genus Ceratostomella Sacc. belonging here. At first Ceratostomella pilifera Wint. was known as the blue-stain fungus. (The commonest blue-stain fungus in the lumber yards of the United States is Ceratostomella pilifera Fries (Winter). It is also found in the forests. (v. Schrenk, Bur. Plant Indus. Bull. 36, 1903; Hedgcock, G.G., Mo. Bot. Gard. Ann. Rept. 17, 1906; and Rumbold, C.T., Phytopathology 19: 597-599, 1929.) Note by translator, C.T.R.) Münch (1907 and 1908) showed that Ceratostomella pilifera simply is a name for a group of fungi consisting of several Ceratostomella species. He also was the first to show the close connection between the Genus Ceratostomella and the Genus Graphium (1907), and described Ceratostomella (formerly known

as Graphium) piceae. Later this was confirmed by Miss Rumbold (1911) for several species of Graphium. (This is a mistake. In one case only has a connection been proved between a Graphium and a Ceratostomella. In the United States Graphium and Ceratostomella appear to live independently and discolor wood as different blue-stain fungi. Rumbold, C.T. Mycologia XXII. No. 4, 1930. Note by translator, C.T.R.)

For a long time there was doubt as to the reason for the discoloration and the way in which it was caused. The hyphae are not colored blue or black, but a light or dark brown. They produce no coloring matter, which can be extracted from the discolored wood. The appearance of the bluing is now believed to be an optical effect. This idea was first advanced by Münch, and is generally accepted. It is caused by the thick tangle of brown hyphae which, "like a finely-divided dark substance in a transparent medium" in the light-colored wood causes the same kind of an optical effect as cigarette smoke in the air or as watered milk. The hyphae commonly live in the parenchyma cells of the wood and bark, fill the cells of the rays with a thick tangle and also invade, though less vigorougly, the tracheids, generally through the pits.

The dead heartwood remains free of the fungi. This case then is not one of true wood-destroying fungi, for there is no appearance either of corrosion or destruction. Experiments by Rudeloff (1897), v. Schrenk (1903) and Münch (1908) have shown that blue stain does not cause a loss of strength or a loss in specific gravity. Rudeloff even determined that it caused an increase in strength.

Although the strength of the wood appears not to be decreased by

blue stain, it is a very undesirable thing in the lumber business, as its appearance makes the wood unusable for many purposes. In general it is the custom for the consumer to allow 10 per cent bluing. It is understood that in every 100 boards delivered, ten may be blue stained. But an intense blue stain on a greater scale will cause the wood to lose 50 per cent of its value.

This loss of value is understandable from a technical point of view. Falck (1916) has noticed that blue-stained wood used as telegraph poles was susceptible to moisture and was unusually disposed to be infected by wood rots. The blue-stained spots soon showed the reddish streaks of wood rots, which are fungi belonging to the Hymenomycetes, closely related to the cellar fungus (Coniophora cerebella). The rot spreads to the sound sapwood and makes the pole unusable in a short time.

For this reason the question of the blue stain caused by bark beetles has an importance for forestry, as yet unknown. The importance for forestry may lie in the possibility of protecting the living trees.

A review of the literature follows:

Münch (1908) conducted experiments which caused him to think that "under certain circumstances blue stain kills living pines;" namely, when the amount of air in the wood allowed the fungus to grow. He remarked also, "The question is complicated in that such trees as are lacking in moisture content, are the first to be attacked by the beetles and killed, and that usually both injuries occur at the same time. Often one cannot decide whether death is caused by the fungus or by the beetles". When living, standing trees are attacked by bark beetles, naturally one is dealing with

certain "primary" bark beetles and certain "primary" fungi. This point of view was expressed lately by Craighead (1928) in experiments with Dendroctonus frontalis Zimm. He came to the conclusion that the combination of blue stain and beetles causes the death of the tree and pointed to the possibility of a kind of symbiosis between fungus and beetle in that the blue stain furnishes the necessary means for the development of the Dendroctonus larvae. These questions will be dealt with later.

Although the simultaneous appearance of bark beetles and blue-stain fungi have often been noticed, and the opinion has often been expressed that possibly the beetles can be the direct carriers of a special fungus, no tests have been made on this subject, as yet. (A beginning has been made in this experimental work by Nelson and Beal. Phytopathology 19: 1101-1107. 1929. Note by translator, C.T.R.)

v. Schrenk (1903) found Ceratostomella pilifera Wint. on standing trees of Pinus ponderosa and believed that the infection took place through the openings made by Dendroctonus ponderosae. The trees were heavily infested with these insects. He believed the wind and not the beetles carried the spores, for his attempts did not succeed when he tried to culture blue-stain fungi from the washings from the beetles or from the contents of their stomachs. He obtained molds and bacteria only. This lack of success may have been caused by v. Schrenk using agar for his plate cultures. The high temperature at which agar hardens, makes it a poor medium for inoculating when it is in fluid form. Also v. Schrenk apparently left out the addition of acid to the medium which would prevent the growth of bacteria and help in the isolation of the fungus spores. As conidia of Penicillium, yeast and bacteria are in the stomach and on the chitin of the beetles; the danger lies in the blue-stain

mycelium being overgrown and suppressed.

v. Schrenk also discovered a species of Ceratostomella in the galleries of Gnathotrichus occidentalis, an Ipid which bores into wood and raises ambrosia. As it did not appear possible that in this case the wind could have carried the spores, v. Schrenk believed that the imagoes may have accidentally carried the blue-stain spores with their ambrosia.

Hedgcock (1906) went further. He found in the galleries of an Ips (not named) a Graphium and connected it directly with the ambrosia of beetles by naming it Graphium ambrosiigerum. He also believed that the Ips played a role as carrier of blue stain and sought to prove it. He put beetles on pure cultures of Ceratostomella, then transferred them to plates of sterile agar. In this way he obtained blue stain growth. But this experiment is not very convincing, as he had no regard for the natural conditions.

Neger (1908) found that the ambrosia fungi of Xyloterus lineatus Ol. was almost always mixed with Graphium piceae. This led him at first to assume that the ambrosia fungus was a form of blue stain which had been modified by the beetles. However, he recognized later that this fungus was a contamination of the ambrosia fungi which belong to the Endomycetes.

It has been frequently observed that Ips is generally accompanied by blue stain and this has led to the opinion that bark beetles play an important role as carriers of blue stain.

The work of Münch (1908) and Craighead (1928) have been cited.

Falck (1916) even pointed out the possibility of the spread of blue stain by the spores and conidia passing through the intestines and the

possibility that their slimy covering may protect them from being digested.

Lagerberg and Melin (1927) believe that the spores are carried on the chitin of the beetles and point to the fact that the stickiness of the Ceratostomella spores makes it very easy for them to stick to animals and be carried by them. Wollenweber and Stapp (1928) point out the very frequent appearance of Graphium ulmi Schwarz, the cause of the Dutch elm disease, apparently a fungus related to Ceratostomella piceae Münch -- in the larval beds of Scolytus scolytus and point out that the elm-dwelling Scolytids possibly were one of the factors in spreading the elm disease.

My own examination of specimens recently infested with Ipides have confirmed these previous observations by showing the almost regular appearance of blue stain or fungi closely related to them.

In order to study the different fungus species, pure cultures were made. They were started from blue-stained pieces of bark or wood taken under sterile conditions or from spores and conidia taken from fruits which develop in a short time on the specimens of bark and wood. The first isolation of these fungi was full of technical difficulties because most of those connected with the Ipides came from a mixture of different blue-stain fungi.

The most important characteristic of the blue-stain fungi which accompanies Ipides is one that all of these fungi have in common; namely, the conidia and spores are covered with a more or less thick covering of slime. This allows them to stick to the chitin of the beetles and they are carried by the insects. A distribution by wind is almost out of the question.

The Ceratostomella species, about which Münch and Lagerberg and Melin have written, have their ascospores embedded in a tough slime in the form of a drop or a tendril on the ostiole of the long-necked perithecia.

This slime is so tough and sticky that it is difficult to dissolve in water. Besides, in many cases there are conidia on the mycelium which, as Münch has observed, are capable to a high degree of budding like yeast, so that when the substratum is unfavorable they can grow over and cover the mycelium. In this they are like certain yeastlike fungi, the so-called "Dematium" or Pionnotes," in the condition of their mycelium. It is not known how often this covering occurs on natural substrata under normal circumstances. These sticky yeastlike conidia can easily be carried by animals. The conidia which appear on the normal aerial mycelium, with the Gladosporium-fascicular form of conidiophore or arranged like the grains on an ear of maise, are loosened from the mycelium readily, and may possibly be distributed by the wind, when they are not held in a tiny drop of slime, and in this form are easily distributed by animals.

The most important form of Ceratostomella conidia which can be born by the winds is that of Endoconidiophora coerulescens Münch, which collect in the mycelial clumps as a fine gray dust, and probably can be easily blown by the wind. The ascospores are not embedded in a tough slime but rather in a watery, easily dried slime which, however, might allow the spores to be carried by insects. This fungus which usually appears on tree blases, was found by me once only in the frass of Ips typographus mixed with other fungi. The Hypomycetes found in the frass of the Ipides have more or less sticky conidia.

The coremia of the species of Graphium cut off conidia into slightly slimy, watery clear or yellowish drops. They flow easily in water

under the cover glasses and when dried become sticky. This fluid covers the head of the coremium so that the conidia which are formed in quantities are collected and held in it.

We find similar conditions with the family of Leptographium, a fungus belonging to the pine blue stains and newly described by Lagerberg and Melin. On these the watery fluid apparently helps to fasten the conidia to the bodies of the insects, while when the fluid has dried out, wind distribution is not out of the question. A new blue stain in association with Ips typographus which I have found on spruce, should be mentioned in this place. It is in many ways similar to the former fungus and apparently belongs to the same family. (Fig. 1.)

Conidia which develop directly on the mycelium without conidiophores, belonging to the blue-stain type Hormonema dematioides Lagerberg and Melin, and which do not possess other fruits, have the ability to bud like yeasts, just as the Ceratostomella conidia. Under certain conditions the whole mycelium can be covered with a white slime.

Other fungi, whose names have not been determined, which belong to the Sphaeropsides, form pycnidia in cultures. Their conidia are suspended in a watery fluid, whth which the pycnidium is filled.

This slimy covering of the spores and conidia makes it possible for the Ipides breeding in the bark to come in contact with the blue stain and as a rule, to be covered with blue-stain spores. In every case in which the imagoes were washed with melted malt gelatine, a larger or smaller number of blue-stain colonies were found together with Penicillium and other organisms. (Table 1.) Also, in the intestines of the beetles, there often

were viable conidia mixed with the food (Table 2). It is a question whether these conidia which have passed through the intestinal tract are not sometimes injured. However, the result of the experiments show that there always were enough spores fastened to the exterior of the insects to infect the frass. Examination for the appearance of certain blue-stain fungi in the Ips gallery showed that the different species of beetles were accompanied with different species of fungi which often were very characteristic .

This phenomenon is explained not only by the different requirements of the beetles, but also of the blue stains on different species of wood and conditions of bark. Just as there are Ipides which typically live in pine or in spruce and only occasionally attack other kinds of wood, so there are blue stains which grow better on pine than on spruce. Experiments of Lagerberg, Lundberg and Melin showed this.

Table 1.--Washings of imagoes plated in malt gelatine

Number	Variety	Yeast -v-	Yeast +	Yeast -	Blue stain +	Blue stain ++	Blue stain +:	Blue stain -	Fungi identified
6	Ips typographus, Mother beetles	6				6			Ceratostomella, Leptographium
5	Ips typographus, Winter sleep		4	1		3		2	.....do.....
6	Ips typographus, Young beetles	6			6				.....do.....
10	Bl. piniperda, Mother beetles	6	4		10				Hormonema
8	C.cinereus, Mother beetles		8		8				Ceratostomella
5	P. chalcographus, Mother beetles	5				5			Ceratostomella, Leptographium Dendrostilbella
2	Ips amitinus, Mother beetles		2		2				Ceratostomella Graph. Leptogr.
2	Dr. autographus, Mother beetles	2			2				Graphium
4	S. scolytus, Mother beetles		2	2	4				.....do.....
12	S. scolytus, Young beetles		7	5			12		Penicillium
2	Ips sutoralis, Mother beetles		2			2			Unidentified Sphaeropsis
2	H. palliatus, Mother beetles		2			2			.....do.....

Table 2.--Cultures of the intestinal contents of imagoes

Number	Variety	Yeast			Blue stain			Fungi identified
		++	+	-	++	+	-	
3	Ips typographus, Mother beetles		2	1		3		Ceratostomella, Leptographium
8	Ips typographus, Winter sleep			8			8	Intestines empty
6	Ips typographus, Young beetles	6			6			Ceratostomella, Leptographium
8	Bl. piniperda, Mother beetles	1	4	3		5	3	Hormonema
4	S. scolytus, Mother beetles		3	1	4			Graphium
6	S. scolytus, Young beetles			6			6	Intestines empty
5	Autographus, Mother beetles	3	2		5			Graphium

Besides, the reqirements of the species of Ipides are very different as regards the condition of the bark. The more primary beetles prefer to attack freshly-felled wood or standing trees which are weakened for some reason, but if there is a lack of material for breeding, they attack perfectly sound trees. The more secondary beetles attack wood felled for some time, whose bark has changed especially as regards its water content.

Nprmally a felled tree trunk is not infected by several species of beetles at one time. Usually the attack of the different species follows in a certain series according to the type of forest. The requirements of the beetles as regards the condition of the tree used for breeding appear also to be very definite.

Conditions for the development of the blue-stain fungi are similar. According to Münch (1908) the water and air content of the wood determines the growth of wood-inhabiting fungi. Careful experiments by Lundberg (Lundberg, Lagerberg and Melin 1927) with different species of blue stain confirmed Münch's statement about these fungi, and showed also that for the different fungi there was a quite different optimum water content of the wood. Those varieties of fungi are always carried by the species of Ipides whose optimum water requirement in the wood and bark is reached during the development of the brood. In this way the advantage is for that fungus whose optimum is reached first. Optimum conditions at the time of

insect attack is not always necessary. The degree of dryness of the wood at the time of attack, therefore, can vary more for the blue stain than for the beetles, with the result that the same fungi can be associated with different Ipides whose requirements for a brood-tree differ not too greatly.

The following discoveries show that these theories can be probabilities. The fungi found on the large amount of material examined, which was infested with Ips typographus are alike in appearance to a remarkable degree, whether collected from the two districts of the Swiss Saxony, the Hinterhermsdorf and Postelwitz or the district Klosterreichenbach, Württemberg. These districts differ in respect to their situation, climate and habitat.

Except for some rather rare admixtures of Ceratostomella piceae Münch, and Ceratostomella pini Münch, which appear to have wide limits to their moisture requirements, and a yellow-headed species of Graphium with a white stalk (fig. 2) which causes a medium amount of blue staining of the substratum, and which does not appear to have been described, there appeared a blue-stain fungus which also had not been described -- one which is a vigorous blue stainer, and whose fruits were the typical ones for the Ips typographus stain. (See Figure 4.) Both of these fungi will be described later. Besides Ips typographus, this latter fungus also accompanied Pityogenes chalcographus on spruce which generally attacks just a little latter than I. typographus. On a wind-fall spruce in

Hinterhermsdorf P. chalcographus had attacked the upper part of the trunk about 10 days later, to judge by the stage of development of the brood, than I. typographus the lower part. Here one found the fungus described above as characteristically going with I. typographus more as a companion fungus. In a short time there appeared on the frass two species of Stilbaceae bearing coremia tipped with clear droplets. One of these did not discolor the substratum. It belongs to the family Dendrostilbella von Höhn and has not been described previously (fig. 3). The other which developed its conidia in chains, I have not named, as the pure culture was lost. (Figure 4.)

A trunk from the same wind-fall region, which had lain for some time, was infested in the lower part by Dryocoetes autographus. In a few days there appeared on the frass in the galleries of the mother beetles the coremia of a Graphium, which barely stained the substratum. It has not been described as yet. The fungus stains the wood a faint lilac brown. (Fig. 5.) The upper part of the tree was infested with Hylurgops palliatus and a vigorous blue stainer was found which belongs to the Sphaeropsides. Fungi have been found in Tharandt belonging to the same family. They were found in the galleries of Polygraphus poligraphus and Orthotomicus suturalis. They appear to require the same high air content of the wood, such as Lundberg stated was necessary for Discola piniecola (Naumov) Petrak. This fungus also belongs to the Sphaeropsidae. However, it was remarkable that these

Sphaeropsodes were found accompanying the Ipides which breed in old, very dry bark.

A pine-inhabiting Ips, Blastophagus piniperda, which appears in Tharandt and the neighboring district Spechtshausen, is accompanied by a fungus which stains the wood and bark green brown. It is almost always the only blue stain to appear on the trap trees which are heavily infested. The fungus apparently is identical with Lagerberg and Melin's Hormonema dematioides. According to these authors the fungus also appears on pine needles. It seems possible, therefore, that it develops during the feeding period of the young beetles on the pine shoots in the young bark and on the needles, and so fits in to the biology of the beetles. It could not be proved that the fungus actually developed there because of a lack of material, as the trap trees were peeled before the emergence of the beetles. A district in Hinterhermsdorf and Ottendorf-Okrilla which has suffered from attacks of the big forest gardener and which has a dry sandy soil in the neighborhood of the Dresden heath, has also been attacked by Blastophagus piniperda which have bred in the standing pines which have been weakened by all kinds of calamities. These beetles have been accompanied by Ceratostomella pini Münch. In Hinterhermsdorf I found Blastophagus piniperda on fallen trees also accompanied by Ceratostomella pini. McCallum (1920) reported from Edinburgh the appearance of C. pini

and C. piceae on standing pines which were badly attacked by Blastophagus piniperda. It is quite remarkable that two such different fungi, a Hyphomycete and an Ascomycete, accompany Blastophagus piniperda. It contradicts the theory that certain Ips carry certain fungi with them which are physiologically adaptable. However, these two fungi of different families, require about the same physiological surroundings; for instance, their requirements in the air content of the wood are the same. According to Lundberg's experiments they were the only fungi in the series which showed themselves quite independent of the oxygen content of the wood. So both of them are capable of infecting fresh and sappy wood, just as Blastophagus piniperda can, when necessary, attack sound trees for breeding. Both fungi are not stopped by the copious flow of rosin in the galleries, which produces a greater or less exclusion of air and can, together with the beetle, cause the death of the tree. It is remarkable that these two fungi are not found together, but one or the other of them accompanies B. piniperda. The reason for this is not apparent, possibly local circumstances play a part.

The result of an investigation of a pine trunk, a wind fall in an old stand of pine in the district of Hinterhermsdorf, was especially interesting. The lower part of the trunk had been infested with Blastophagus piniperda. The insects had emerged already. Broad patches under the

bark were covered with the perithecia of Ceratostomella pini. The upper part was heavily infested with Ips amitinus and in the smooth bark there were several galleries of Pityogenes chalcographus. Both of these beetles really are spruce inhabitants. The larvae of both beetles were grown or pupating, and in the oldest I. amitinus galleries there were a few young beetles. The fungus-flora was varied. Five different kinds of blue stain were isolated from the I. amitinus infested part of the log; two kinds of Graphium which were not identified and which grew on pine wood in Erlenmeyer flasks quite as well as on spruce; also C. pini which prefers to grow on pine rather than spruce, and two typical spruce fungi, C. coerulea Münch and Leptographium Lundbergii Lagerberg and Melin, these two latter fungi being the most prominent. It is remarkable that a pine which had been accidently infested with spruce beetles should act as a conserver of the pine blue stain. However, both fungi belong to forms which are adapted by their sticky conidia and spores for being spread by insects. An explanation for this might be the assumption that this case is one of an attack by a strain of I. amitinus which has bred for generations in pines and so in time has adopted pine fungi.[1] This assumption may also be an explanation for the fact that beetles which usually seek pines for breeding, were

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1 For choice of breeding place by polyphagous beetles, see Hopkins, U. S. Dept. Agr. Progr. of W. 1917. Craighead, J.A.R. 22 1921.

given a very good opportunity to use the heavy windfall of spruce in this region of the mountains.

This account of the blue-stain flora of the Ips galleries is by no means complete. Probably the number of species is much greater. Only the principal forms are described here.

In this connection I wish to state again the possibility that the Scolytids spread the Dutch elm disease. The specimens of dying Ulmus montana from Loschwitz which I examined were heavily infested with Scolytus scolytus and Scolytus multistriatus. The bark and wood were permeated by the mycelium of Graphium ulmi, which developed innumerable coremia in the pupal chambers and on the frass in the larval faces. There were two other fungi with it, which were not identified. Apparently the larval excrement is a favorable substratum. Of these fungi Graphium ulmi is the principal one which has the characteristics of an entomological fungus. The fruit carries a honey-colored, water drop in which large numbers of conidia are collected. These, as well as the innumerable conidia which develop on the mycelium, tend strongly to bud like yeasts so that the pionnotes-like condition of the mycelium in culture is very prevalent. This characteristic mycelium and its yeastlike budding conidia helps one to recognise with some ease the Graphium mycelium which develops in cultures made from the contents of the intestines.

In the intestines of larvae and imagoes and on the outside chitin of the beetles are found the Graphium ulmi conidia as well as the spores of other fungi. Isolation of these colonies were made from poured plate cultures made with the washings of insects and intestines. (Figure 6 and Tables 1 and 2.)

It looks as though the beetles which swarm in the spring and whose food are the young sprouts of the elm, carry conidia stuck to their bodies. They can even infect perfectly healthy trees whose bark is not suitable for breeding, as the fungus can penetrate the tree in any place. (Wollenweber and Stapp 1928.) Unfortunately exact experiments could not be made to prove whether the young beetles really carried conidia, as the material had dried out during the winter and the greatest part of the fungi had died. The emerging beetles were almost entirely infected with the hardy Penicillium spores. Their mycelium quickly overgrew all other organisms in the cultures.

As a footnote to these observations, a fine article by Betrem appeared in Holland in 1929 about the spread of the Dutch elm disease by Scolytus scolytus. She describes essentially the same facts, which are confirmed by my observations. Her work also lacks experiments with the emerging young beetles, and these are necessary for a complete solution of the problem.

The regular appearance of blue stain or closely related fungi in the galleries of the bark breeding Ips raises

the question whether there is a reciprocal action between fungus and insect, in other words a symbiosis. Aside from Craighead's important exposition, which can apply only to primary insects, the feeding of the Ips larvae or beetles raises the question whether there is a cultivation of fungi by the Ips which is similar to the ambrosia cultivation of the wood breeders.

So far there are just two cases of bark-breeding Ips known to have a relationship with fungi.

According to Wichmann (1927) Blastophagus minor Htg.) lives in symbiosis with fungi, which are carried by the mature beetles, spread through the galleries and furnish nourishment for the larvae, so that they are able to complete their development in a remarkably short time. When Blastophagus minor chances to infest spruce, the fungi do not grow well, the development of the larvae slows down, and the beetles haunting the galleries are hungry. The results of my own experiments are not conclusive, as I had only old material from which the young beetles had flown. The material was heavily blue stained, and Ceratostomella pini and C. coerulea were identified as well as species of yeasts. Whether these fungi are symbionts of Blastophagus minor must remain a question.

Another case of Symbiosis with bark-breeding Ips is the ambrosia cultivation of Xyleborus cryptographus Rtzb. This insect belongs systematically to the ambrosia beetles and like them cultivates ambrosia in the bark.

In order to decide whether other bark dwellers also show a variety of symbiosis, it is necessary to examine closely the relations between fungus and beetle; in other words, the spread of the fungi in the galleries and their ecological importance for the insect. My examination was carried on with Ips typographus and Blastophagus piniperda. There was a great deal of material in all stages of development to be had in trunks of fallen trees.

The spores of the fungi mentioned as appearing with I. typographus and B. piniperda, are carried by the mature animals, which have wintered in galleries infected with blue stain, or are carried by insects which have stayed under or in the bark which have blue stain spores sticking to them.

Table 3.--Cultures of frass from egg galleries

Number	Variety	Yeast development			Remarks
		++	+	-	
10	Ips typographus	10			Blue stain
10	Bl. piniperda	10			....do.....

Table 4.—Washings of Ips eggs cultured in malt gelatine

Number	Variety	Yeast development ++	Yeast development +	Yeast development −	Remarks
65	Ips typographus		65		Occasional blue stain
30	Bl. piniperda		30		A little blue stain
26	Dr. autographus	26			The most blue stain
50	P. chalcographus		50		Occasional blue stain
14	Ips amitinus		14		
10	O. laricis	10			No blue stain

The spores are brushed onto the bark of the tree occupied by the brood and find a suitable substratum for development. They grow frequently in the frass in the pupal chambers where the beetles, by their industry, have plugged up the niches. (Table 3 and 4.) The fungus spreads from the pupal chambers where the frass apparently favors germination, into the galleries, and most of the wood fungi then grow very rapidly in the direction of the fibers of the wood and bark; as there is less resistance to overcome from cell walls, they penetrate less quickly centripetally into the wood through the rays, and grow the slowest in the direction directly tangential to the fibers. When the tree is first attacked the surroundings apparently are not favorable for the fungus. The larvae bore into the bark ahead of the fungus, as one finds no pieces of

mycelium or spores in the stomachs of the young larvae. Later as the bark begins to dry, the blue stain spreads. Discoloration of wood and bark begins when the hyphae become brown and thick, which happens about 3 weeks after the mother beetles borein. It is remarkable that a streak about 1 mm. wide remains white right and left of the brood chamber. The fungi growing from the egg niches have spread peripherically on one side only. Apparently they are stopped from spreading in this direction by the vigorous growth of the yeasts in the neighborhood of the brood chamber. The yeasts flourish vigorously on the sappy substratum and are advantageous to the brood at its start.

The spread of the fungi in the galleries of typographus and of piniperda vary somewhat.

The procedure with Ips typographus is somewhat as follows: At first the growth is greatly inhibited while the larvae gnaw into the unblued bark, there vigorous mycelial growth begins. The bluing is gemerally done by the fungus which accompanies typographus. The spread of the fungus along the fibers is rather quick, so that the larvae gnawing in an acute-angled direction to the fibers and the younger larvae, are overtaken by the fungus. For spreading at right angle to the fibers, the fungus generally uses the larval galleries filled with excrement. The rapidity of the growth is such that a partially grown larva which gnaws across the fibers eats just about at the boundary of the mycelium. When the larvae are grown, at the latest, at the time in which the white grown larvae will not take more

nourishment, the blue stain spreads over all the galleries, across the fibers and, while the pupae lie motionless in the pupal cells in large numbers, the blue stain forms the conidiophores characteristic for the typographus fungus.

In the case of Blastophagus piniperda, only its relation to Hormonema dematioides was examined. I had no young material to study of Blastophagus piniperda accompanied by Ceratostomella pini. Here the procedure is different in that the fungus does not reach the boundaries of the galleries during the time the larvae are feeding, and the larvae are constantly gnawing white bark, free of fungi. The growth of the fungus and its spread in the galleries may greatly depend on outside factors, for instance on temperature and rainfall, but as a rule it goes as I have described.

The question arises whether the beetles regularly carry blue stain fungi which have an ecological importance for the larvae and imagoes.

As has been stated, the blue stains do not attack cellulose or cellulose-like substances, so these fungi can not be regarded as agents for the consumption of cellulose. If there is by any chance a food symbiosis, it is one of using the contents of parenchyma cells situated at a distance from the Ips galleries and transporting the food stuff by the growth of the fungus to places where it can be reached by the insect.

The above observations show that Hormonema dematioides can not be a factor in the feeding of Bl. piniperda. The

stomachs of the larvae are free of mycelial fragments or conidia, as a rule, and very seldom could conidia be identified in the stomachs of the mother beetles.

The company of the blue stains regularly seen with Ips typographus very probably is advantageous for feeding the insects. The beetles and larvae feed on the blue stain fungi. The viable blue stain conidia and mycelium found in the intestinal tracts of the older larvae and imagoes show this. The difference in the rate of growth of the fungus in the galleries when parallel to the fibers or across them, will allow one to compare the larvae of the same brood, if there is not too thick an insect invasion. One can compare the larvae mining in an acute angled direction to the direction of the fibers forming brood galleries in blued bark, or mining more directly across the fibers and in bark which is very little or not at all blued.

The different ages of the larvae must be considered also. A distinct difference in the development or the growth of the two sets of larvae can not be noticed. Therefore one can assume that the mycelium of the fungi is not necessary, though it may be carried by the insect and incidentally serve as food in the normal development of the larvae.

This was confirmed by observation of two broods in a spruce log held in the laboratory. The out surfaces were covered with paraffin to prevent evaporation and remained free from blue stain, perhaps because of unfavorable conditions, possibly the too rapid drying of the wood. In spite of conditions which were not normal for the larvae either, they

developed in 4 to 5 weeks into pupae of the usual size.

Though it appears that the larvae of Ips typographus apparently do not need fungi as food for normal development, it still is a question whether the fungus mycelium in the pupal cells is not advantageous or even necessary for the young beetles, or the galleries made by maturing beetles are not mined more quickly in bark permeated by mycelium than in that free of fungous growth.

To prove this point, pupae were kept on moist filter paper in Petri dishes until they transformed; they were then transferred to large Petri dishes and placed part of them on pieces of bark having pupal cells covered with fungi and part placed on sound bark. The pieces of bark were renewed several times while the experiment was being run, as soon as fungous growth — in this case Ceratostomella piceae — appared. In the first set, the freshly emerged beetles buried themselves in the pupal cells and began to graze on the fungus mycelium. In the second set, the beetles hid themselves between the pieces of bark and began gnawing on the sound bark. No difference could be detected in the browning and the vitality of the beetles after being watched for 6 weeks. One must conclude that an obligatory food symbiosis like the cultivation of ambrosia does not exist between these two kinds of beetles and blue stain. Whether these conditions are similar for other Ipides is difficult to determine, for example, for Crypturgus cinereus, an occasional parasite of Ips typographus whose larvae mine the

strongly blued bark, and whose imagoes are thickly covered with blue stain spores. Apparently there is as little obligatory symbiosis between Ips amitinus and blue stain as with I. typographus.

A late brood of mother beetles produced just two larvae in a spruce log which was held in the laboratory, the beetles having originated as previously mentioned in heavily blued pine material. Though blue stain did not appear, they grew normally. It is probable that the Ipides which breed in the bast region do not depend on fungus for food, but can incidentally make use of it.

The principle development of the fungus in the direction of the fibers' length explains perhaps the fact that Blastophagus minor, whose egg galleries run horizontally and whose larvae gnaw principally in the direction of the fibers, can regularly change over to using fungus food, while their near relative Bl. piniperda, whose egg galleries are parallel to the direction of the fibers and whose larvae gnaw across or at an angle to the fibers, remain independent of fungus food. Perhaps this fact is important, namely that Bl. minor breeds in the smooth bark and is more subject to drying out so that the symbiotic fungi have a better chance to develop than they have in the thick bark, where as a rule Bl. piniperda breeds.

It is hard to say whether the more primary Ipides show a symbiosis with blue stain in that their combined attack on the brood tree is deadly, as Craighead has shown in the case of Dendroctonus.

At any rate this question must be considered, whether this partnership in general may not be shown by the fungus making possible such conditions as permit the beetles to live. According to Münch's experiments it is possible for blue stain to kill trees if conditions are right, so it may be possible for it to support the beetles when killing the trees. Descriptions of Ips attack on fallen trees show the beetles as the pioneers for the fungi rather than vice versa, as the mining larvae first bring about the death of the tree and hasten the drying out of the bark. It can hardly be possible that the Ips blue stain invasion of standing trees differs in procedure from that on fallen ones. Certainly the fungus helps greatly in killing the trees, but in those cases observed the advantage has been greater for the fungus than for the insects, if one can speak of give and take between fungus and insects.

The circumstances for the elm scolytids are slightly different, as they are the carriers of the conidia of Graphium ulmi. This fungus is strongly pathogenic and can easily penetrate the sound tree from infection points, i.e. from where the beetles feed, and so weaken the tree as to make it easy for the scolytids to attack the bark. But this case is possibly a special one. Here one may possibly speak of a symbiosis with the meaning described by Craighead, not however meaning single individuals, but meaning the whole species.

Other insects which are known to live in the Ips galleries appear to make more use of the fungus flora of the Ipides. Aside from the fungus-mites which can make themselves so unwelcome in the Petri dish blue stain cultures, and the true species of fungus midges which according to Münch can live entirely on blue stain, there are several Coleoptera which are frequently in the bark beetle galleries, which live partly on the excrement of the Ipides that were infected with fungi and bacteria, and which also appear as enemies of the Ips larvae. Among the Coleoptera are many Cucujids, Cryptophagids, Lathridiids, Mycetophagids and Colydiids. For instance, Perris (1877) has described the larvae of Cucujids, Loemophloeus testaceus which are met in company with Dryocoetes capronotus and are considered enemies of Ips larvae: "It lives also on their excrement, but it can develop perfectly and transform in the galleries of the xylophagous when devoid of the inhabitants."

Certainly in this case as in others, the mycelium permeating the excrement, plays a role as a source of food and substitute for animal protein. Perris says of the larvae of the Mycetopagids, Litargus connexus, they live "amidst the debris left by other larvae, principally the scolytids but chiefly on that bark which has been covered with certain mycelium."

So it seems to be among the above mentioned beetle families which accompany the Ipides, that the real users of

the Ips fungi are seen, especially as all these species are nearly related to those which are purely fungus eaters.

b) Yeasts Carried by Ipides

The second group of fungi which accompany the Ipides have an unusual position as they are capable of living in the interior of the stomach of the insects. A series of mounted slides of cross sections through the stomachs of the larvae show yeasts in large numbers, sprouting vigorously. They are found principally on the wall of the stomach in the neighborhood of the secreting cells but also on pieces of food which are being digested. The yeast cells are not changed even at the end of the intestine and as a rule viable yeasts can be obtained from the fresh feces of the larvae. This is not a case apparently of inner symbosis, in which the symbiont is cultivated in the stomach and also is digested. There is still a possibility that the insect uses the fungus engyme or secretion.

Before I describe the relations of the yeasts to the Ipides and the expeeiments made to free the animals of their yeasts, I would like to give in brief form the general characteristics of the yeasts belonging to the Ipides.

The yeast cells in the stomach of the larva are oval, average 2-1/2 to 3 μ long and 2 to 2-1/2 μ wide, but can, when growing under favorable conditions in gelatine cultures, growing to twice this size. They have a coating of slime, like the blue stain spores, which adapts them for carriage by insects and also possibly protects them from being digested.

There are vacuoles in the interior which increase as the cell eggs and when badly nourished, fill the cell lumen. There are also oil droplets in the plasma and scattered chromatin granules.

If these yeasts are sown in malt gelatine in Petri dishes, they form colonies of yeast, which show characteristic growth forms. At first they form almost round colonies. After a few days some colonies start developing small hyphae, which are formed from cells which chanced to lie at the periphy; while others increase simply by budding. These latter colonies grow partly in circular form, partly with thick, dense core and looser, wavy exterior (fig. 7). They have an average diameter of about 1 mm., but in one case there was a variety of yeast from the intestine of a larva of Scokytus pruni which formed giant colonies on the surfacesof the substratum.

The mycelium-forming colonies reach a diameter of 1 to 10 mm. The growth of the hyphae is limited, it cases when the hyphae reach a certain length or is stopped in some way. The conidia which form on the mycelium increase in number by vigorous budding so that finally the whole mycelium is encased in yeast. (Fig. 8.)

The cultures made from the intestinal contents of different Ips larvae show an extraordinarily large number of different forms of yeasts. It happens that in the different cultures one or two forms occur in greatest number, the others appear apparently by chance.

The different species of Ips seem usually to carry certain kinds of yeasts, some of which develop characteristic growth, others can be distinguished simply by rapidity of growth and diameter of hyphae.

It is very hard to find characteristics which will distinguish the cells of the different varieties of yeasts, for the cells in these colonies show great variation in form and size.

Several photographs and drawings may help to show the general appearance of the growth of the yeasts of the Ipides.

The cultures from which these colonies were derived were grown on the same medium -- 15 per cent gelatine, 20 per cent beef extract, 1 per cent Maltyl (dry malt extract sold by the Gothe Co.) -- as yeast growth depends to a great extent on the nutrient medium. The drawings are somewhat diagramatic and only show the mycelium growing in the middle layer of the medium.

Figure 9 shows a yeast-fungus found regularly accompanying Ips typographus. The species of yeasts which accompany Ips typographus will be reported in greater detail in the part describing the fungi.

The species of yeast which appears most frequently in the larvae of Ips amitinus resembles the yeast-fungus associated with I. typographus. It however grows more rapidly and forms a larger mycelium. A yeast which has been found associated with Pityogenes chalcographus also is a rapid grower and has a much branched mycelium. (Fig. 10.)

A yeast from the larvae of Scolytus scolytus develops a mycelium of irregularly woven hyphae which have conidia scattered along them. (Fig. 11.)

Figure 12 shows a species of yeast which has been isolated almost exclusively from the stomach contents of Blastophagus piniperda. It develops a much branched mycelium but as a rule just a few cells situated on the edge of the colonies germinate and produce hyphae. The figure shows mycelium five days old. By the time it was nine days old the hyphae were thickly covered with yeast. One might say that the mycelium had yeasted.

A species of yeast was isolated from several larvae of Dryocoetes autographus which was very characteristic. It developed a branched mycelium on which the conidia appeared in round balls. The mother colony did not form a single round center but looked more like a mulberry, as it was formed of grouped small round colonies (fig. 13). Besides this, there was a growth which looked like the I. typographus yeast and a species of yeast with short mycelium and a large center of yeast whose conidia formed chains by continuously sprouting at the tip. (Fig. 14.)

One among other yeast-fungi isolated from larvae of Ips laricis developed a sturdy unbranched mycelium on which the conidia developed in fascicled form, budded and formed short linear yeast chains. (Fig. 15.)

All these fungus forms, in spite of several differences, show many common characteristics and behave exactly alike in culture. There is no formation of gas , the gelatine is not liquified if there is no bacterial contamination. Experiments must be made to knowwhether these fungi are capable of decomposing cellulose or cellulose-like substances. The yeasts grew on pieces of sterilized conifer bark and wood in Erlenmeyer flasks. They grew in a smeary covering, penetrated the parenchyma cells and vessels on the surface, and appeared to macerate the bark easily.

Their behavior in older cultures and the factors which influence their growth are described later when the yeasts of Ips typographus are described. Everything said there applies generally for the Ipides' yeasts.

Many of these yeast-fungi, for example those that accompany Ips typographus, Dryocoetes autographus, Orthotomicus laricis and Pityogenes chalcographus develop hat-shaped endospores in gelatine cultures, such as are found in several species of the Saccharomycete-family Willia and as found by Buchner and Heitz in the symbiosis of Tetropiinae and Spondylinae. (Fig. 19, a and b.)

In order to study the spread and appearance of yeasts, cultures were grown in cleared malt gelatine. They were made under the most sterile conditions possible. The intestinal contents of Ipides in all stages were used, also fresh and old feces and frass from egg galleries. The washings of eggs and imagoes were also plated in malt gelatine.

The results were as follows:

The infection with yeast of the frass in the galleries is made by the adult beetles, which are covered with sticky yeast as well as with blue stain spores. (Table 1.) There are occasional yeast cells in the stomach of the imagoes, however, not so frequently and not so many as in the stomachs of the larvae, so possibly the yeast cells continue their growth on the feces. (Table 2.) At any rate the spread of the yeast is certain as every gallery investigated showed frass infected with yeast.

When the beetles bore into the bark of freshly attacked trees, the yeast can find suitable nutrient in the bark of the primary galleries, especially in the moist frass with which the beetles generally plug up the egg chambers. (Table 3.) They quickly increase there by budding, so that the parent beetles which pass along the gallery are smeared with yeasts and can infect fresh egg niches. The yeast cells have been found even in the sawdust which the beetles make at the entrance holes. But principally the egg embedded in the gnawings is covered with yeast, so that when the larva crawls and bores into the bark, it is pretty sure to carry yeast cells along in its intestinal tract and so infects the bark. (Table 4.)

The conditions are the same for those species which lay their eggs in rows without egg niches (Dryocoetes

autographus) or loosely in heaps in the gallery (Orthotomicus laricis.) The transported yeasts multiply in the primary gallery on the sparse shavings to which the eggs are glued, and on the slimy secretion of the glands which have covered the surface of the eggs, and the beetles with their hairy bodies smear them over the egg niches and their neighborhood.

The infection of the larvae can be easily followed from eggs embedded in gelatine. The hard shelled eggs of Blastophagus piniperda, Orthotomicus laricis, Dryocoetes autographus also of Pityogenes chalcographus are not killed in gelatine. The larvae emerge through a slit in the shell. The larvae can be seen with their heads often covered with pointed hairs pressed against the shell wall. They nod and bend and stretch. The emerging larvae take yeast with them from the neighborhood of the eggs; they gnaw galleries several centimeters long in the gelatine and the entire length of their gallery shows a heavy growth of yeast.

If the young larvae are infected in the gallery, they continually infect the old feces with yeast which they push behind them. The yeast grows over the surface of the neighboring wood and bark particles. The old larval excrement is more or less permeated with yeast. (Table 5.)

The number of yeast cells found in the lumen of the stomach varies greatly. Sometimes the larvae carry comparatively few, at other times the stomach lumen, especially when

the stomach is filled, will show in mounted sections an overwhelming number of yeasts. (Table 6.)

Table 5.--Shake cultures of old larvae feces

Number	Variety	Yeast development			Remarks
		++	+	-	
2	Ips typographus	2			Heavily blue stained
6	Blastophagus piniperda	3	1	2	Blue stain in the oldest feces
2	Scolytus scolytus	1		1	Graphium ulmi + unknown fungus
3	Scolytus pruni	3			Unidentified fungus
2	Hylurgops palliatus	2			Blue stain
4	Pityogenes chalcographus	3		1	Blue stain
8	Ips amitinus	7		1	Blue stain

Table 6.--Shake cultures of the intestinal contents of larvae

Number	Variety	Yeast development			Remarks
		++	+	-	
15	I. typographus, mature larvae	6	9		Heavily blue stained
5	I. typographus, young larvae		5		No blue stain
16	Bl. piniperda	8	7	1	.....do.....
8	Hylurgops palliatus	7	1		Blue stain
2	Polygraphus poligraphus	2			.....do.....
3	Pityogenes chalcographus	3			.....do.....
6	P. chalcographus from pine	3	1	2	1 Slightly blue stained
9	Ips amitinus from spruce	5	2		
10	Ips amitinus from pine	4	3	3	Heavily blue stained

The numberof yeasts in the stomachs is greatly reduced when the animals are hungry; when the larvae are shedding or about to pupate, the yeasts can entirely disappear. However, in one case 28 yeast colonies were isolated from the rather empty stomach of a larva which had starved for 24 hours, an indication that the yeast cells are protected from digestion. The stomachs of pupae are relatively free of yeasts; in one case only 7 viable yeast cells were isolated.

On the walls of the pupal niches the yeasts which are last excreted by the larvae grow and are eaten by the young beetles. There are, for instance, very many yeasts in the stomachs of the young typographus beetles and the exteriors are also heavily smeared with yeasts. In consequence the young beetles when emerging carry their yeasts with them. It may be that they fly to feed on the young parts of plants, on the new host for over-wintering or for breeding.

The over-wintering of yeasts must take place outside of the insects for the stomachs of over-wintering beetles have been found free of yeasts.

The association of the bark breeding Ipides with yeast fungi shows many similarities to the symbiosis of the Cerambycids with Saccharomycetes. There are, however, some important differences.

The method of transmission to the new generation through infection of the larva by mouth, by contact and boring

in the wood are the same. But in this case the yeasts remain free in the lumen of the stomach while in the case of the Cerambycids the yeasts are held by special organs in the middle intestine, but are also found in the lumen of the stomach.

Several larvae of Rhagium mordax Deg., which I examined, which had over-wintered in the open with empty stomachs, were found with intestinal organs filled with yeasts. Here also the over-wintering was in the body of the animal.

The Ipides lack the complicated means for transmittal in the genital apparatus of the females which Buchner and Breitsprecher have described for Cerambycids and Anobiides. The transmission by the beetles is for the Ipides quite uncertain, it is left entirely to chance.

The symbionts of the Ceramhycids are in ways similar to the yeast fungi of the Ipides. This was shown in comparative cultures of yeasts taken from several bark beetles (Tetropium sp., Rhagium inquisitor L., Rhagium mordax Deg. and Leptura rubra L.). According to the formation of spores the yeast of the Ipides stand near systematically to the symbionts of the Tetropiinae and Spondylinae in which the hat-shaped spores were found in the special apparatus of the female which is used for transmitting the spores. In general the appearance of the cultures made from the contents of the Ips intestines is not as uniform as cultures from the symbiont organs of the Cerambycids.

In spite of these differences the conjecture remains that the yeasts of the Ipides in a way could be considered to show an inner symbiosis, in that in some way the enzymatic ability of the fungi is used. The investigations were made to test the importance of yeast for the Ipides, especially to determine whether or not the larvae can develop normally without them.

Naturally it was very seldom that yeast-free larvae were found. There were 80 larvae investigated, either the intestinal contents or the excrement in their galleries being examined. Of these therewas just one larva of Scolytus scolytus, Blastophagus piniperda, Pityogenes chalcographus and three larvae of Ips amitinus (the two latter found on pine), which were free. (Table 6.) But even these findings are open to error, because of varying conditions in the cultures.

In order to eliminate error, an attempt was made in the laboratory to obtain from sterilized eggs of Ips typographus, larvae free of yeasts. The result was not satisfactory as the artificial breeding of larvae is very difficult.

It was difficult to disinfect the eggs. The yeast cells bud deep in the slimy covering of the eggs, and they are so well protected against harmful fluids that one must use fairly strong substances to obtain a complete sterilization, and when doing so, the embryo generally was killed or so injured that the larva died soon after it emerged.

The disinfection of the eggs was attempted with different concentrations of hydrogen peroxide, sublimate and Chinosol used for different lengths of time, but generally the disinfection was not perfect. From 20 eggs handled in this way only 7 young beetles developed, and of these one only was free of yeasts. The other larvae died shortly after emerging. Apparently there were other conditions which influenced these results (ventilation, conditions of the bark) for the checks in which unsterilized eggs were used, also were not successful as the larvae died as soon as they emerged.

Disinfection was obtained by washing the eggs for 2 minutes in 1 per cent mercuric chloride solution, but this usually killed the embryo in the egg. Twenty-four eggs were used in this experiment, and just 2 developed. However, these grew in 3 weeks into normal, yeast-free larvae.

The experiment was made in the following way:

The eggs were carefully removed from the egg niches, and sawdust removed. The eggs were put in mercuric chloride solution for 2 minutes and washed in a great deal of tap water, then put with a flamed instrument into artificial egg niches. These were cut into a piece of spruce in the following way:

Bark which had been washed in sublimate solution and tap water had slashes cut with a flamed knife, making strips about 6 mm. wide and 10 cm. long. These formed the artificial galleries and egg niches in the bast. After the eggs had been placed in the niches the gallery was plugged with cotton and wrapped with strips of tinfoil. It is questionable whether

these conditions were natural enough for the welfare of the brood. However, the two larvae which survived and developed normally showed that the yeasts were not absolutely necessary. No yeasts were found on them either outside, in the stomach or the excrement. However, a mold had grown into their galleries, so that these larvae did not develop absolutely free from fungus contact.

It was noticed that these larvae and young beetles which emerged and fed in solitary state, separated from contact with their kind, gnawed almost exactly in the direction of the fibers.

The results of this experiment were confirmed by cultivating in the laboratory a brood which developed normally, although the conditions were unfavorable for the development of yeasts. Fifty larvae developed normally, which showed when examined that in 20 the stomach lumen was free of yeasts, in 15 a few colonies only developed, 5 showed a fairly heavy growth of yeast, and only 8 were heavily infected.

It therefore appears that the yeast fungi in the stomachs of the Ipides at least have little biological importance for the insects.

They do not appear to be pathogenic, for two sickly larvae showed fewer yeasts, than the average number found in the healthy.

Although there is a possibility that whatever circumstances help the fungi also help the insects, I am inclined to

think they are not necessary for an optinial development of the larvae, that it is rather a kind of commensalism. There also is the possibility that under certain conditions the presence of species of yeasts in the insect stomach can lead to symbiosis, such as exists with the Cerambycids and Anobiinae. The bark breeding Ipides in general appear to be independent of fungus organisms.

General Remarks About Wood and Bark Nutriment

It remains to be seen whether a symbiosis with bacteria exists. The fundamental differences between wood and bark makes it thinkable that the bark breeding Ipides in contrast to the wood dwelling could not live on a cellulose medium without an adjusting symbiosis.

The wood breeders are offered the food which must be converted from the starch and oil contents of the rays and wood parenchyma cells; these are comparatively scarce in the wood.

The bark breeders feed principally in the bast, a region rich in proteins, in the bark parenchyma and cambium layer, also with cell contents rich in nitrogen, reserve stuffs easily converted by digestive enzymes.

The bast layer has comparatively little cellulose and cellulose incrustations -- substances which compose most of the wood. The larva of the bark-brood mines through the young vessels in case it invades the splint and attacks the thickened cell walls only of such sclerenchyma fibers or

stone cells as pass through the phloem. But these wall thickenings are comparatively unimportant in comparison with those in the wood.

The small amount of strengthening substance in the bast layer shows in its weakness in comparison with the wood. It offers little resistance to the mandibles of the animals which can obtain enough food from the consumption of sufficient quantities of plant tissue to insure their development in a short time.

Further, it should be noted that the wood vessels in the region where the wood-brood lives, serve principally for the transportation of water, except in the case of deciduous trees at the beginning of the growing season. On the other hand the sieve tubes in the region where the bark-breeding Ipides feed are filled with the concentrated assimilation products which are being transported from the leaves to the cambium and roots. To a lesser degree this transportation takes place in felled trees also. According to Münch (Büsgen-Münch 1927) the sap in the sieve tubes of trees contains about 25 per cent of dissolved or suspended substance, principally raw sugar, in which other substances are mixed, i.e. protein and their decomposition products. Starch granules are also found in the plasma of the sieve tubes. Then too the bark contains more nutrient salts than the wood.

It is quite possible when considering these differences of the sources of food to believe that the bark-

breeding Ipides can live without the use of the contants of ungnawn cells and of cell walls.

Descriptions of Some of the Fungi Found During the Course of the Experiments

a) Leptographium penicillatum nov. spec.

This fungus has been found regularly accompanying Ips typographus and causing blue stain. It was found in standing trees. It has not as yet been described. The typical brush-like conidiophores appear in the pupal cells in most cases a few days after the beginning of the pupal resting stage. The walls of the cell are covered with a thick felt of the fungus. (See fig. 1.) The conidiophore consists of a shaft, a single row of cells, made up of about 7 long, thick-walled dark brown cells, on the top of which is a brush-like head on which the conidia develop. The length of the stalk varies between 300 to 450 u, its diameter is about 7 u. The top cell divides into two less often into 3 branches, which themselves divide into 3 less often into 2 branches. It develops dichotomous and trichotomous branching. (Fig. 16.) The last branches grow out into non-septate hyphae 2 u in diameter, on whose tips conidia develop successively, forming a fascicle. They are hyaline, when young a long oval, when ripe curved like a sickle, and have a length of 11 to 12 u and an average diameter of 3 to 3.5 u. The entire conidiophore is between 450 and 600 u long. The conidia-bearing

brush is covered with a drop of clear, watery fluid. In the pupal cells the conidiophores stand so thickly that the individual drops flow together and later when the solution dries the brushes are bound together by a dirty white, sticky mass of conidia.

Put in malt gelatine, the conidia germinate very readily. They become round, oval, increase in volume, form one or more large vacuoles, and under favorable conditions produce a germ tube at both ends. Under especially unfavorable conditions the conidia usually bud like yeasts rather than produce mycelium. For instance, when they are sown too thickly, mycelial growth is entirely suppressed and they develop little heaps of budding yeast-like conidia, whose sprouts continually become smaller.

The young hypha is 2 u in diameter, at first seldom septate, later it becomes 5 u thick, heavy walled, brown and gradually the cells grow shorter.

About 4 days after inoculation, long or short branches develop on the mycelium, on whose tips fascicles of conidia develop which look like Ceratostomella pini Münch. (Fig. 17.) The conidia are hyaline, oval and very different in size and shape, varying between 6 and 14 u in length and 4 and 6 u in width. These differences are possibly caused by the differing degrees of ripeness and the position in which the conidiophore is growing. They possess in a high degree the faculty of budding like yeasts and call to mind the Pleonectes-condition of fungi.

In a situation such as is caused by unfavorable food conditions (overfeeding with malt?) the substratum is hardly stained, because the mycelial growth is hindered and the hyphae do not turn brown at all or brown very slowly.

Conidia do not develop on brown mycelium, instead the brush-like conidiophores do. In normal cultures, which show a gray-brown aerial mycelium, there appears after a short time, a dark blue-black discoloration of the substratum. Gelatine is liquified in both cases. The fungus grows about as rapidly as Ceratostomella piceae Münch.

The fungus belongs, systematically, to the Hyphomycetes and particularly to the Stachylides, to which Lagerberg and Melin, 1927, gave the new genus Leptographium. It differs from Leptographium lundbergii Lagerberg and Melin, which is a typical pine fungus, principally by the long stalk of the conidiophore, which contains 7 to 8 cells, and by the shape and size of its conidia, further by the fact that it is found only on spruce. The method of branching of the conidiophore and the arrangement of conidia on the mycelium are very much alike. This fungus is placed in the genus Leptographium, Lagerberg and Melin. It is closely related apparently with Hantzschia phycomyces Auerswald, described by Wollenweber and Stapp (1928, Table 3, G) whose conidiophore is very similar to those of the new Leptographiums. Saccardo has placed this fungus in the family Graphium Corda, wrongly I think, and as Wollenweber has already pointed out. In spite of the two fungi

showing great similarity, they are different in manner of growth. The conidiophores are "somewhat thickened at the base and provided with root-like strands" (according to Rabenhorst, Crytogamenflora Vol. 1, Part 9, p. 354). In the case of this fungus the shaft develops directly from the cell of a hypha and diminishes at the base rather than increases. The length of the oval conidia of Hantzschia is given as 3 to 4 u, a size which does not correspond to the ripe curved conidia of Leptographium. Lastly, the place in which Hantzschia phycomyces was found "in a cellar on oaken casks," shows that it is a fungus which appears to differ physiologically from the blue stain of coniferous lumber.

The identity of the Leptographiums with Scopularia venusta Preuss, which belongs to the Stachylides and for which Preuss made a genus Scopularia, can be shown by the description only, not by specimens or drawings. Lagerberg and Melin have already pointed out the possibility of the identity of these fungi with Leptographium lundbergii. My new variety shows a still greater resemblance with Scopularia as the conidiophore in this case consists of a long shaft of several cells, while that of Leptographium lundbergii is made of fewer and short cells. Preuss' illustration shows a very peculiar, unnatural branching of the shaft which is hard to comprehend, and which is in every way different from the branching of Leptographium. However, the correctness of Preuss' drawing has been doubted already by Saccardo (Syll. Fung., Vol. IV, p. 330). As

Scopularia venusta apparently is a blue stain, and also found on pine wood, the possibility still remains that the two fungi are the same. As Preuss has not given the size and no specimens of his Scopularia exist, the identity of the two fungi cannot be proved now. I therefore have given the new fungus the name Leptographium penicillatum nov. spec. The description is as follows:

(Translated from Latin)

Leptographium penicillatum Grosmann nov. spec.

Young mycelium hyaline, later dark brown. The hyphae penetrate wood tinging it blue, young hyphae develop conidia. The fertile stalk is erect, long, simple, brown, the tip divides like a brush into hyaline filaments, the head covered with a liquid is about 600 u in diameter. The conidia borne at the tips, curved, hyaline 11-12 x 3-3.5 u.

Found in the wood of Piceae excelsae. Associated with Ips typographus, Pityogenes chalcographus. Synonym perhaps Scopularia venusta Preuss.

b) Graphium pycnocephalum nov. spec.

Another blue staining fungus found very frequently accompanying Ips typographus is a variety of Graphium, for which so far no description has been found. The Graphium appears in the pupal cells a little later and less thickly than the conidiophores of the previously described variety. The hyphae vary between 2-4 u in diameter, at first hyaline, later light brown and frequently septate. They produce an intense bluing of the substratum. Conidia are not produced on the mycelium, but the

mycelium tends to form clamydospores when food conditions are unfavorable. The coremia have a white shaft. The head is covered with a light colored, slimy, yellowish drop of fluid. The fruiting bodies are 700-1000 u long. The shaft has a length of about 550-700 u and a width of 50-80 u. The head is about 150-200 u high and 150-300 u wide. The shaft is woven out of thin hyaline hyphae which are thickly branched in the region of the head forming a very thick club- or hat-shaped head. The hyphae bear on their tips fascicles of longish oval conidia. These are hyaline about 6 u long and 2.2 u wide. When germinating the conidia become roundly oval, and develop germination tubes at both ends, without any budding like yeasts. On agar the fungus does not develop coremia; abnormal graphia developed on sterilized pieces of spruce twigs in Erlenmeyer flasks. Perithecia were not seen. The fungus was found on frass in galleries of Ips typographus in Klosterreichenbach (Württemberg) Postelwitz and Hinterhermsdorf (Sachs. Schweiz). (See fig. 2.)

(Translated from Latin)

Graphium pycnocephalum Grosmann nov. spec. Mycelium very thin, without conidia, the fertile stalks are about 1 mm. high, erect, hyaline. The tips yellow. The conidia are borne on the tips in fascicles, are longer than wide, measuring 6 x 2.2 u.

Grows in the wood of Piceae excelsae. Associated with Ips typographus.

c) Graphium adustum nov. spec.

This variety of Graphium has been isolated from wood infested with Dryocoetes autographus found in Hinterhermsdorf. It produces its coremia on the freshly loosened pieces of bark in the abandoned egg galleries. (See fig. 5.) It faintly discolors its substratum with shades of lilac-brown. The septate hyphae are hyaline, later light brown, and have a diameter of 2-4 u. The young hyphae develop a large number of round-oval conidia, the mycelium-conidia are 5 u long and 2.5-3.5 u wide, and appear on the ends of long or short branches of the hyphae in fascicular forms. (Fig. 18a.) They can bud like yeasts. The graphium stalks develop on agar or gelatine media. They vary in height between 400-700 u. The shaft is colorless at first, later turns a yellow brown in the middle while both ends remain light. It is 300-500 u long and 50-150 u thick. The head is covered with a dark yellow drop of liquid, which later turns a brown black. The height of the head is 75-100 u, the width 150-300 u. The conidia which develop on the branched hyphae of the head are a round oval, about 2.2 u thick and 3 u long. The development of mycelium often precedes the yeast-like budding of the conidia. Perithecia did not develop.

(Translated from Latin)

Graphium adustum Grosmann nov. spec. Mycelium hyaline later strongly tinted. The hyphae produce conidia.

Stalks are 400-700 u high. At first they are hyaline bearing yellow droplets, later they and droplets are colored a dark yellow. Conidia are nearly round, 3 x 3.5 u.

Grows in wood of Piceae excelsae. Associated with Dryocoetes autographus.

4) Dendrostilbella hyalina nov. spec.

The coremia of this fungus develop in large numbers together with other unidentified varieties of the Stilbaceae in the egg galleries of Pityogenes chalcographus. The specimens were collected in Postelwitz in the Switzerland of Saxony. (Fig. 3.) They were cultivated in Erlenmeyer flasks on pieces of spruce bark with larva frass. The conidiophores remained abnormal and sterile when cultivated on artificial media. The coremia are 400-500 u long and absolutely clear as glass. The brush-like head is covered with drops of colorless liquid. The shaft is 300-350 u long and 25-35 u wide. The height of the head is 120-150 u and the width 60-75 u. The branched hyphae in the head are relatively thick. The conidia arehyaline, oval 3 u long and 2-2.2 u wide. When the conidia germinate, before they develop a mycelium, they form a row of large roundish oval cells. (Fig. 18b.) The hyphae are colorless and 2-5 u thick. They often form round giant-cells with a diameter of 9 u. It was not determined whether conidia developed on the hyphae. The substratum was not colored.

(Translated from Latin)

Dendrostilbella hyalina Grosmann nov. spec.

Mycelium and coremia white. Coremia formed like a brush about 500 u high, bearing colorless droplets. Conidia borne singly. 3 x 2.2 u.

Grows in wood of Piceae excelsae. Associated with Pityogenes chalcographus.

e) Experiments with the Species of Yeasts found with Ips Typographus

In gelatine shake cultures of typographus material two forms of yeasts usually appear. These were studied in pure culture. A part of the colonies developed through budding into spherical colonies which increase to a diameter of about 1 mm. (Fig. 19, a.) Variety a.

Another part also developed at first into spherical colonies, which however produced mycelium after about 48 hours. As has been described before, this develops from yeast cells which lay at the periphery and is composed of hyaline septate hyphae growing vigorously in radial directions. (Fig. 19, b.) Variety b. In malt gelatine cultures the hyphae are not branched as a rule; in peptone -- cane sugar gelatine -- they branched a little. On malt gelatine growing under the most favorable conditions, the mycelial colonies had a diameter of about 4 mm.

The hyphae are about 1.5-2 u thick. They produce many conidia, at first just beyond the septum of a hypha,

later along its entire length. These conidia on the hyphae behave like yeast cells and begin at once to bud abundantly. The first ones which are produced directly on the hyphae are larger and longer than their budding daughter cells, so that they produce the effect almost of being small conidiophores. As the production of conidia for a time is always just beyond a septum, the conidiophores at first are arranged almost concentrically in mother colonies. Later the hyphae are covered for their entire length by heaps of yeast cells. (Fig. 8 and 9.)

Both varieties produce endospores in gelatine about 14 days to 2 weeks after the colonies have stopped spreading. These are large and hat-shaped, such as are found in several Endomycetes and several varieties of the Saccharomycetes family Willia, Hansen. A third form, variety c, (Fig. 19, c) was isolated but once. Germination resembled the second variety, number b, except that the mycelium was much thicker. The hyphae had a diameter of 3-3.5 u. The mycelium spread over malt gelatine for a distance of 8 mm. Spore production was not seen.

As has been stated, it was difficult to show the principal differences of these three varieties in the size and form of their yeast cells. The cells of variety a were on the whole larger and rounder, those of variety b, smaller and more oval, and those of variety c, relatively large and long. However, it is almost impossible to give exact measurements.

Tests were made of the reactions of the different colonies of different media, to see if there were three varieties of yeasts. The experiments were to show also whether outside factors influenced their manner of growth.

The position of the colony in the medium had a great influence on their manner of growth. Colonies lying below the surface of the medium developedmore and stronger mycelium than those growing on the surface, or those which reached the surface of the medium in their growth. The yeasts of the colonies growing in the medium scarcely increased after the formation of mycelium, while those colonies on the surface developed yeast cells so abundantly that the mycelium which did grow was entirely covered with yeast, and at most just the tips of the hyphae showed. The yeasts of the surface colonies grow in the shape of a flat or semi-spherical, wavy, buckled colony (Kalotte).

Experiment 1.— A test of both yeasts in regard to their growth in or on the substratum. Yeast cells of strains a and b, taken from pure cultures, were sown by the same method beneath the surface of the substratum. This was done by allowing the inoculated layer of gelatine to harden and then pouring over it an uninoculated layer.

The result was that all the colonies of strain a showed yeast budding, while strain b developed mycelium. Further, the behavior of the colonies depended on the depth to which they were submerged when sown. If they were near the surface the development of yeast and mycelium was sparge, for

both kinds of colonies were reciprocally limited. The colonies remained small, such hyphae as developed remained short and very few cells in a colony developed hyphae (fig. 8), while most of them did not produce hyphae at all.

Experiment 2.--Yeast cells of strain a and b were each sown very thickly in gelatine medium. Result: Both cultures showed no development of mycelium. They formed very small dot-like colonies.

Experiment 3.--From these two cultures yeast cells were taken as inoculum and sown less thickly in gelatine. Result: Strain a did not develop mycelium; strain b produced mycelium in 90 per cent of the colonies.

The composition of the medium has a big influemce on the growth of the yeasts. It was noticed that colonies hanging on to the frass which was suspended in the gelatine, grew unusually well. Malt appeared to increase the development of yeast in the core of the colony and the covering of the hyphae with yeast.

Cane sugar combined with peptone or with sodium hippurate, while it hindered the formation of yeast and favored the formation of hyphae, did not cause an unlimited growth of mycelium. This same result has been obtained by Schwarz, 1924, when cultivating the symbionts of his cochineal insects.

Experiment 4.--To test the effect of different nutrient media on strains a, b, and c, (See table.) they were inoculated on media as follows:

Nutrient medium 15% gelatine + 4% citric acid	Mycelial development			Production of spores		
	a	b	c	a	b	c
2% meat extract	-	+	+	+	-	-
1% meat extract + 2% malt extract	-	+	++	+	+	-
4% malt extract	-	++	+++	+++	++	-
8% malt extract	-	++	++	+++	++	-
2% plum juice	-	+	+	++	+	-
6% cane sugar + 1% sodium hippurate(hippuricum)	-	+	++	-	-	-
10% cane sugar + 1% peptone	-	+	++	+++	+	-

The result of this experiment shows that the tendency to form yeast or mycelium is dependent in many cases on environmental conditions and undoubtedly there are different strains of yeasts which accompany Ips typographhus.

This has been confirmed by an experiment with asci. Strain a develops 2, 3, and 4 spores in a cell, while with strain b, usually 2 spores are found in an ascus. As has been stated previously, strain c has never been noticed to develop spores.

The systematic position of these yeasts which accompany the Ipides is difficult to determine, though they apparently are closely related. Probably they belong to the Saccharmycetes, in that their spores, in such cases where spores are produced, are produced in asci which are developed

from vegetative cells. Copulation has never been observed.

Strain b certainly is identical with a fungus which has been described as Endomyces bisporus by A. Beck, 1923. The writer isolated the fungus from the galleries of Ips typographus and from dead larvae. She did not investigate closely the relation between the fungus and the insect, but left the question open "whether the fungus lives parasitically on the live insects or is saprophyte which settles on the dead insects." A. Beck believed this fungus which possesses the same kind of spores as certain Endomycetes must be placed in this family for several reasons, but especially because of the relatively strong development of mycelium. This characteristic does not necessarily prove it belongs to the Endomycetes, in my opinion. Relatively well developed mycelium are found in the life cycle of several true Saccharomycetes, for instance in Saccharomyces Marxianus Hansen. Moreover, there are known to be quite a number of Saccharomycetes which possess the ability to ferment sugar, just like the yeasts of the Ipides. The asci do not develop on the mycelium only as with the Endomycetes, but also in the center of the yeast colonies and in the heaps of conidia on the mycelium where they often form whole chains of asci. No reason exists for placing this variety in another family than the one to which strain a belongs. Strain a obviously belongs to the Saccharomycetes, as it does not form a single hypha like for example, Endomyces capsularis which stands next to the yeasts.

Graphium adustum nov. spec. develops on a poor substratum. No connection of any kind has as yet been found between the yeasts and the blue stain fungi. The yeasts produce a small amount of mycelium even under the most favorable conditions. Gelatine medium does not liquify as it does in blue stain cultures. The hyphae always remain hyaline, never brown. They never form aerial mycelium or conidiophores. The cultures also have a smell typical of yeasts and in no way resemble the characteristic smell of the blue stain cultures. Also those yeasts, which so far have not been observed forming spores, apparently are Saccharomycetes, which since they behave alike when growing in culture, are closely related, systematically, to the spore-forming yeasts of the Ipides.

f) Results of an Investigation of the Symbiosis Problem

1. The bark-breeding Ipidæ play an important role as carriers of blue stain and related fungi.

2. The fungi transferred by the insects have characteristic criteria, spores and conidia, which are embedded to a greater or lesser degree in slimy liquids.

3. The different fungi which are transferred by the Ipidæ have the same requirements for living as the insects. As a rule the same varities of fungi appear with the same varieties of Ipides.

4. From the experiments which have been made with certain varieties of bark-breeding Ipides, the insects in general do not appear to be dependent on the blue-stain flora

for life; whenever the blue stain fungi are at hand, however, the larvae and imagoes consume the spores and pieces of mycelium.

5. Most of the yeasts are found in the stomach lumen of the Ips larvae. They resemble the fungi isolated from the yeast organs of certain Cerambycids.

6. The yeasts are transferred by the mother beetles, the infection by the larvae is by their creeping and boring into the bark.

7. Experiments with Ips typographus have shown that the yeasts are not absolutely necessary for the development of the insects, they may simply be regarded as universally present.

8. Several fungi have been described which were found in the course of the experiments.

Bibliography follows. See the original paper for this and illustrations. Paper appeared in Zeitschrift für Parasitenkunde. 3:57-102. Illustrated. D. 17, 1930, No. 1.